IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Dario NERI et al.

Examiner: Alana M. Harris

Serial No.: 09/194,356

Group Art Unit: 1642

Filed: September 2, 1999

Title: ANTIBODIES TO ED-B DOMAIN OF FIBRONECTIN, THEIR CONSTRUCTION AND USES

REPLY

Mail Stop
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The examiner alleges that the two Japanese publications anticipate the claims because they prima facie disclose antibodies binding to the ED-B domain of FN. Applicants stand by their stated position that both of these documents do not support an anticipation rejection of the claims at least because they clearly are not enabling for production of an antibody binding to the ED-B domain of FN. Nevertheless, in order to expedite prosecution, applicants have obtained a letter signed by Professor Sekiguchi, the main inventor of the two Japanese applications and in whose laboratory the work was performed underlying the two Japanese patent applications.

The letter is self-explanatory and establishes that the deposited antibodies reported in each of the two Japanese applications, in fact, do not bind to the ED-B domain of fibronectin. This situation is similar to that for the prior art antibody of record, BC-1, as explained by Dr. Sekiguchi, where an antibody appeared to bind to the ED-B domain but by further testing was shown not to so bind.

Clearly, the two Japanese publications do not anticipate the claimed subject matter and do not achieve preparation of an anti-EDB antibody nor in reality state how to do so.

The underpinning of all of the obviousness rejections is an allegation that one or the other of the Japanese documents discloses an antibody which binds to ED-B and/or discloses how to prepare such an antibody. Since this has now been established as incorrect, it can be seen that all obviousness rejections are untenable for at least this reason.

Incidentally, the antibody, OAL-pF115 mentioned by the examiner, is not an antibody of the "invention" of JP '195. Rather, it is a commercially available control antibody. Note page 20, third full paragraph, where, with respect to such antibody, it is stated "pFN established by Sigma as the antigen." Furthermore, for the record, no basis is given in the office action for the allegation that, as a purified monomer, the prior art specific binding member would inherently have the stated dissociation constant, even if it had had the alleged specificity. In any event, this point is rendered moot by the Sekiguchi letter.

As for the sequence disclosure matter raised in item 3 of the office action, the specification was amended on page 24, lines 15-17, 22 and 24 with identifying SEQ ID NOS in the amendment filed December 28, 2000. See, pages 1-2 of the amendment.

Based on the foregoing, it can be seen that all claims are in condition for allowance.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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August 29, 2005

TO WHOM IT MAY CONCERN:

RE: JP (A) H2-76598 and JP (A) H4-169195

This letter was written to endorse the novelty and importance of the phage antibodies against the EDB domain of human fibronectin, which had been elegantly produced by Dr. Luciano Zardi and his collaborators. I am currently the Professor of Division of Protein Chemistry, Institute for Protein Research, Osaka University, Suita, Osaka, Japan, and have long been studying the structure-function relationships of extracellular matrix proteins, particularly fibronectins and laminins, during the past 25 years. My major interests on fibronectins have been focused on their molecular heterogeneity arising from alternative RNA splicing. I have witnessed the significant contributions by Dr. Zardi in this research field: He first identified the EDB domain, the most interesting alternatively spliced domain in fibronectin and demonstrated that expression of EDB-containing fibronectins is regulated in an oncodevelopmental manner. In the years 1986-1990, I held a position as Assistant Professor at Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, Japan, and conducted a series of experiments on the oncodevelopmental expression of EDA- and/or EDB-containing fibronectins at both RNA and protein levels. One of my major efforts during the period was to produce monoclonal antibodies recognizing alternatively spliced domains of human fibronectins, i.e., EDA, EDB, and IIICS. For production of anti-EDB antibodies, I produced two separate immunogens: one was a mixture of synthetic peptides EGIPIFEDFVDSSVGY and YTVTGLEPGIDYDIS, both modelled after the amino acid sequence of human EDB domain, and another was a recombinant EDB domain expressed as a Protein-A fusion protein. These immunogens were sent to Drs. Hisanobu Hirano and Sadahito Shin at Otsuka Assay Laboratories, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan, with whom I collaborated to produce anti-EDB monoclonal antibodies. Our efforts yielded two monoclonal antibodies, OAL-CF525 and OAL-TFN-01, both specifically recognizing EDB-containing human fibronectins. We submitted Japanese patent applications for these monoclonal antibodies on September 14, 1988 (OAL-CF525; no. 63-230458) and October 31, 1990 (OAL-TFN-01; H2-295820), which were subsequently published as, respectively, JP (A) H2-76598 on March 15, 1990 and JP (A) H4-169195 on June 17, 1992.

The evidence by which we claimed these monoclonal antibodies were the antibodies recognizing EDB-containing human fibronectins was as follows. The first antibody OAL-CF525 was produced using the EDB-derived synthetic peptide and screened for positive reactivity with intact EDB-containing human fibronectins purified from the conditioned medium of SV40-transformed human fibroblasts (WI-38VA13) and for negative reactivity with human plasma fibronectin lacking the EDB domain. These screening strategies, in combination with the use of the EDB-derived synthetic peptides as an immunogen, were the basis of our claim that the resulting antibody recognizes the peptide sequence present in the EDB domain of human fibronectin. Similarly, OAL-TFN-01 was produced with an immunogen, the Protein-A fusion protein containing the EDB domain flanked by short segments of neighbouring III-7 and III-8 domains, and obtained after screening for positive reactivity with EDB-containing cellular fibronectins secreted by SV40-transformed human

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fibroblasts (WI-38VA13) and negative reactivity with plasma fibronectin. Although we did not have concrete evidence for the direct binding of these monoclonal antibodies to the EDB domain at that time, we believed that these results were sufficient to claim that these antibodies were capable of specifically recognizing EDB-containing fibronectin isoforms and were therefore directed to the EDB domain.

However, in 1992, Dr. Zardi reported an important observation regarding the antibody that had been considered to recognize the EDB domain. I should stress that the first antibody recognizing the EDB-containing fibronectins was produced by Dr. Zardi. The anti-EDB monoclonal antibody, BC-1, originally reported in 1989 (Carnemolla et al., J. Cell Biol., 108, 1139-1148, 1989), recognizes only EDB-containing human fibronectins, and therefore had been believed to recognize the EDB domain itself. Dr. Zardi showed in 1992 that the antibody BC-1 did not bind to the EDB domain per se but recognized a conformational epitope residing in the preceding III-7 domain, which was cryptic unless the EDB domain was inserted between III-7, and III-8 domains. Dr. Zardi's report reminded me that our monoclonal antibodies might have similar problems regarding their epitopes.

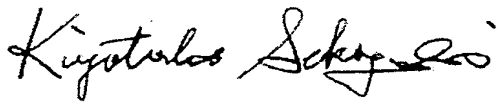
Accordingly, we performed additional experiments to see whether one of our monoclonal antibodies, i.e., OAL-TFN-01, was indeed directed to the EDB domain or its neighboring region which was cryptic without EDB insertion. We examined whether OAL-TFN-01 could bind to the recombinant EDB domain produced as a glutathione S-transferase (GST) fusion protein without any flanking domains and whether the GST-EDB fusion protein could inhibit the binding of the antibody to EDB-containing cellular fibronectins, using conventional ELISA assays. The experiments were done more than 10 years ago and the results were not available anymore at this moment, but I was sure that the antibody did not show any specific binding to the GST-EDB fusion protein, nor did it show any decrease in its binding to EDB-containing cellular fibronectins in the presence of the GST-EDB fusion proteins. These results led us to conclude that OAL-TFN-01 was not directed to the EDB domain per se, but rather recognizing the epitope residing in the region other than EDB, whose reactivity with OAL-TFN-01 was cryptic unless the EDB domain was inserted between III-7 and III-8 domains, as was the case with Dr. Zardi's monoclonal antibody BC-1.

More recently, upon reminder by Dr. Zardi, I performed another experiment in which I examined whether OAL-CF525, an antibody raised against synthetic EDB peptide, might have the same property regarding its epitope recognition. I obtained from Dr. Zardi a recombinant fibronectin fragment encompassing III-7 through III-9 domains with an insertion of EDB between III-7 and III-8 domains (designated "7B89") as well as a recombinant EDB fragment containing only the EDB domain and examined whether the reactivity of OAL-CF525 to "7B89" was inhibited by the recombinant EDB fragment to see if the antibody really recognizes the epitope within the EDB domain. I found that binding of OAL-CF525 to "7B89" was unaffected by the presence of increasing concentrations of the recombinant EDB fragment, although the reactivity of L19 phage antibody (now known as an authentic EDB antibody; a gift from Dr. Zardi) with "7B89" was clearly inhibited by the recombinant EDB fragment in a dose-dependent manner. These results indicated that OAL-CF525 is not directed to the EDB domain per se, but rather recognizes an undefined epitope produced by the insertion of EDB between III-7 and III-8 domains, as were the case with Dr. Zardi's BC-1 and our OAL-TFN-01. In addition, our results confirmed the conclusion that Dr. Zardi's L19

phage antibody (Pini et al., J. Biol. Chem., 273:21769-21776, 1998) is an authentic anti-EDB antibody recognizing the EDB domain itself.

The amino acid sequence of the EDB domain has now been shown to be stringently conserved among different species, exhibiting 100% identity among human, rat, and mouse. Thus, it is quite reasonable to assume that the antibody recognizing the EDB domain is naturally removed during the process of antibody production, otherwise such EDB antibodies should attack the host, resulting in severe autoimmune diseases. Therefore, production of anti-human EDB monoclonal antibodies in mice should be practically very difficult, often leading to isolation of pseudo-anti-EDB antibodies recognizing conformational epitopes within neighboring domains resulting from insertion of EDB domain. My recent experience with OAL-SF525 reminded me the difficulty in production of authentic anti-EDB antibodies. To avoid these intrinsic problems, it was very wise to employ the phage antibody technology, with which one can avoid such problems closely associated with production of mouse anti-EDB antibodies. I believe that the innovation introduced by Dr. Zardi and his collaborators is an excellent achievement that allows us to investigate the physiology and pathology associated with EDB-containing fibronectins in humans.

Sincerely,



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Enclosure: Curriculum Vitae of Kiyotoshi Sekiguchi

CURRICULUM VITAE

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Date of Birth: October 5, 1950, Tomakomai, Hokkaido, Japan

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Osaka University, Suita, Osaka, Japan 1978 (Biochemistry)

Positions:

1978-1979	Postdoctoral Fellow, Institute for Protein Research, Osaka University
1979-1981	Postdoctoral Fellow, Fred Hutchinson Cancer Research Center, Seattle, WA
1981-1984	Associate, Fred Hutchinson Cancer Research Center, Seattle, WA
1984-1986	Assistant Professor, Department of Pathobiology, University of Washington, Seattle, WA
1984-1986	Staff Scientist, Fred Hutchinson Cancer Research Center, Seattle, WA
1986-1990	Assistant Professor, Fujita Health University, Toyoake, Aichi, Japan
1990-1991	Associate Professor, Fujita Health University, Toyoake, Aichi, Japan
1991-1992	Chief, Pathobiology Division, Research Institute, Osaka Medical Center for Maternal and Child Health, Izumi, Osaka, Japan
1992-1998	Director, Research Institute, Osaka Medical Center for Maternal and Child Health, Izumi, Osaka, Japan
1998-present	Professor, Institute for Protein Research, Osaka University, Suita, Osaka, Japan
2000-present	Project Director, Sekiguchi Biomatrix Signaling Project, Japan Science and Technology Agency, Nagakute, Aichi, Japan

Memberships:
American Society for Cell Biology (1981-present)
American Society for Biochemistry and Molecular Biology (1986-present)
The Japanese Biochemical Society (1986-present)
The Japanese Cancer Association (1986-present)
Japan Society for Cell Biology (1986-present)
Japanese Society for Tissue Engineering (1997-present)
The Japanese Society for Connective Tissue Research (1998-present)
Protein Science Society of Japan (2001-present)

Editorial Boards:
Editorial Board, The Journal of Biochemistry (1994-1995)
Associate Editor, The Journal of Biochemistry (1996-1999)
Editorial Board, Cell Structure and Function (1999-2000)

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